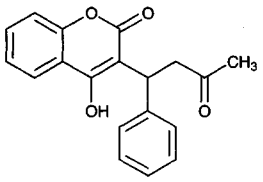
Warfarin



Molecular formula: $C_{19}H_{16}O_4$
Molecular weight: 308.33
CAS Registry No.: 81-81-2, 129-06-6 (sodium salt)
Merck Index: 10174
Lednicer No.: 1 131

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL 2 M HCl, 100 μL 10 μg/mL diclofenac in 10:90 isopropanol: hexane, and 5 mL ether to 0.5 mL human plasma. Shake vigorously for 10 min and centrifuge at 4° at 1200 g for 10 min. Remove a 4 mL aliquot of the organic layer and evaporate it to dryness using a centrifugal vacuum evaporator at 50° for 30 min. Reconstitute residue with 200 μL isopropanol:hexane 10:90 and inject a 40 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 Chiralcel OD
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Isopropanol:acetic acid:hexane 18:0.5:81.5
Column temperature: 25
Flow rate: 1
Injection volume: 40
Detector: UV 312; circular dichroism detector (Model J-720, Jasco, Tokyo)

CHROMATOGRAM

Retention time: 13.1 (R), 25.3 (S)
Internal standard: diclofenac (9.6)
Limit of detection: 20 ng/mL (R), 40 ng/mL (S)

OTHER SUBSTANCES

Simultaneous: hydroxywarfarin
Noninterfering: acetaminophenon, aspirin, baraprost, captopril, cilostazol, diltiazem, dipyridamole, disopyramide, furosemide, ibuprofen, ketoprofen, metoprolol, ticlopidine
Interfering: indomethacin

KEY WORDS

plasma; chiral; pharmacokinetics

REFERENCE

Takahashi,H.; Kashima,T.; Kimura,S.; Muramoto,N.; Nakahata,H.; Kubo,S.; Shimoyama,Y.; Kajiwara,M.; Echizen,H. Determination of unbound warfarin enantiomers in human plasma and 7-hydroxywarfarin in human urine by chiral stationary-phase liquid chromatography with ultraviolet or fluorescence and on-line circular dichroism detection, *J.Chromatogr.B*, **1997**, *701*, 71–80.

SAMPLE

Matrix: blood
Sample preparation: Condition a Bakerbond C18 SPE cartridge with 2 mL MeOH and 2 mL 1 M HCl. Mix 300 μL plasma with 2 mL 1 M HCl. Add to the SPE cartridge, wash with 2 mL HCl, elute with 2 mL MeOH. Concentrate the eluate to 25 μL under a stream of nitrogen, dilute with 85 μL mobile phase, centrifuge at 16000 g for 15 min. Inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Pirkle covalent (R, R) Whelk-O 1 (Regis)
Mobile phase: MeCN:0.5% glacial acetic acid 40:60
Flow rate: 1
Injection volume: 100
Detector: UV 313

CHROMATOGRAM
Retention time: 13.6 (S), 15.8 (R)
Internal standard: ethylwarfarin (16.5 (S), 18.5 (R))
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES
Simultaneous: amiodarone, aspirin, diclofenac, furosemide, ibuprofen, isosorbide dinitrate, lisinopril, phenytoin, tolbutamide

KEY WORDS
plasma; chiral; SPE

REFERENCE
Henne,K.R.; Gaedigk,A.; Gupta,G.; Leeder,J.S.; Rettie.A.E., Chiral phase analysis of warfarin enantiomers in patient plasma in relation to CYP2C9 genotype, *J.Chromatogr.B*, **1998**, *710*, 143–148.

SAMPLE
Matrix: blood, tissue
Sample preparation: Condition a silica Sep-Pak SPE cartridge also containing 2 g sodium sulfate (?) with 5 mL MeOH and 5 mL cyclohexane. Mix 3 mL blood or crushed tissue with 1 mL 20 μg/mL IS, adjust to pH 3-4 with 0.5 M sulfuric acid, extract three times with 10 mL MeOH: chloroform 10:90 (Caution! Chloroform is a carcinogen!). Evaporate at 40°, re-dissolve the residue in 5 mL cyclohexane, sonicate and centrifuge three times. Remove a 5 mL aliquot of the top layer, evaporate at 40°. Reconstitute the residue in 5 mL cyclohexane. Add to the SPE cartridge, elute with 5 mL MeOH, evaporate at 40°, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES
Column: 200 mm long μBondapak C18
Mobile phase: MeOH:0.8% acetic acid 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 4.5
Internal standard: N,N-diphenylbenzidine (9.3)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: bromadiolone, brodifacoum, coumarin, coumatetralyl

KEY WORDS
SPE; plasma; heart; lung; liver; kidney; spleen

REFERENCE
Park,S.W.; Seo,B.S.; Kim,E.H.; Kim,D.H.; Paeng,K.-J. Purification and determination procedure of coumarin derivatives, *J.Forensic Sci.*, **1996**, *41*, 685–688.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Mix 500 μL plasma with 30 μL MeOH, 50 μL 6 M hydrochloric acid and 3 mL diethyl ether, vortex for 30 s, centrifuge at 2000 g for 10 min, evaporate ether layer in a 45° water bath under a stream of nitrogen. Reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot. Urine. Mix 200 μL urine with 20 μL 6 M hydrochloric acid, 40 μL MeOH and 8 mL diethyl ether, vortex for 30 s, evaporate to dryness in a 45° water bath under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak C18 (plasma), 150 × 3.9 5 μm Resolve Spherical C18 (urine)

Mobile phase: MeCN:buffer 38:62 (Buffer was 10 mM potassium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 229 em 389

CHROMATOGRAM
Retention time: 9
Internal standard: warfarin

OTHER SUBSTANCES
Extracted: furosemide
Simultaneous: quinidine, sulfamethoxazole
Noninterfering: carbamazepine, cimetidine, diazepam, disopyramide, fluvoxamine, furosemide metabolite, meclofenamate, metoclopramide, phenobarbital, phenylbutazone, phenytoin, ranitidine, theophylline, trimethoprim

KEY WORDS
plasma; warfarin is IS

REFERENCE
Abou-Auda,H.S.; Al-Yamani,M.J.; Morad,A.M.; Bawazir,S.A.; Khan,S.Z.; al-Khamis,K.I. High-performance liquid chromatographic determination of furosemide in plasma and urine and its use in bioavailability studies, *J.Chromatogr.B*, **1998**, *710*, 121–128.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 205.2

CHROMATOGRAM
Retention time: 20.358

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk

Sample preparation: Mix 100 μmol warfarin, 1 mg 4-(N,N-dimethylamino)pyridine, 200 μL triethylamine, and 1 mL dichloromethane, completely flush the vessel with nitrogen and cap it. Add dropwise 45 μL (S)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (ACROS, Fairlawn NJ) via a syringe. Stir at room temperature for 3 h. Add 20 mL dichloromethane and 30 mL 100 mM pH 6.0 phosphate buffer. wash the organic layer twice with 30 mL portions of phosphate buffer, extract aqueous layer twice with 30 mL portions of dichloromethane. Dry the combined organic extracts over sodium sulfate, filter through a 2 g zirconia. Evaporate solvent in vacuum, re-suspend residue in THF. Inject a 0.1 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 2.5 μm Hp-C/ZrO2 (The carbon-coated zirconium particles were prepared by chemical vapor deposition. Pass heptane through a zirconium dioxide particles in a tube furnace at ca. 700° and at reduced pressure (15-10 Torr) for 1.5 hour. Rinse particles with THF, extract particles with toluene in Soxhlet extractor.)
Mobile phase: THF:water 45:55
Column temperature: 30
Flow rate: 1.0
Injection volume: 0.1
Detector: UV 254

CHROMATOGRAM

Retention time: 6.2, 6.9 (enantiomers)

OTHER SUBSTANCES

Also analyzed: 4-chloroamphetamine, phenylalanine

KEY WORDS

chiral; derivatization; details of column preparation

REFERENCE

Jackson,P.T.; Kim,T.-Y.; Carr,P.W. Diastereomeric selectivity of carbon-coated zirconia reversed-phase liquid chromatographic media, *Anal.Chem.*, **1997**, *69*, 5011–5017.

SAMPLE

Matrix: eggs
Sample preparation: Add 2 g anhydrous sodium sulfate to 5 g egg white or yolk. Extract twice with 15 mL acetone:diethyl ether 90:10. Homogenize for 5 min, centrifuge at 10000 g at -8° for 5 min, evaporate combined supernatants to dryness at 40°. Reconstitute dried extract with 3 mL MeCN. Wash twice with 3 mL hexane and centrifuge at 10000 g at -8° for 5 min. Discard hexane phase, evaporate MeCN phase to dryness under a stream of nitrogen in a 40° dry bath. Reconstitute with 1 mL mobile phase and filter it through a 0.45 μm filter. Inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 4.6 × 4.0 5 μm LiChroSpher 100 RP-18E
Column: 125 × 4.6 5 μm LiChroSpher 100 RP-18E
Mobile phase: MeOH:ammonium acetate triethylamine buffer 62:38 (Prepare buffer as follows. Mix 3.85 g ammonium acetate, 2 mL glacial acetic acid and 2 mL triethylamine in water, adjust to pH 5.2 with glacial acetic acid and make up to 1 L with water.)
Flow rate: 1.0
Injection volume: 50
Detector: UV 281

CHROMATOGRAM

Retention time: 3.1
Limit of detection: 6 ng/g (white); 5 ng/g (yolk)
Limit of quantitation: 20 ng/g (white); 15 ng/g (yolk)

KEY WORDS

eggs; yolk; white

REFERENCE
Pouliquen,H.; Fauconnet,V.; Morvan,M.-L.; Pinault,L. Determination of warfarin in the yolk and the white of hens' eggs by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *702*, 143–148.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 250 μL 1 M HCl to 500 μL microsomal incubation, extract with 3 mL MTBE, evaporate the organic layer under nitrogen, reconstitute the residue in 100 μL MeCN: water 50:50, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 Novapak C18
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM acetic acid adjusted to pH 4.8 with ammonium hydroxide.)
Flow rate: 1
Injection volume: 120
Detector: Radioactivity, Inus β-Ram using Inus Tru-Count scintillation fluid at a flow rate of 5 mL/min

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver

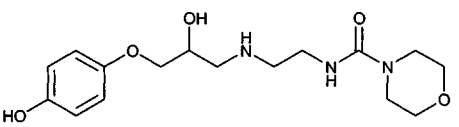
REFERENCE
Obach,R.S. Nonspecific binding to microsomes: Impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol, *Drug Metab.Dispos.*, **1997**, *25*, 1359–1369.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm), dilute the filtrate with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:10 mM pH 4.7 acetate buffer 50:50
Detector: UV 214

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

Xamoterol



Molecular formula: $C_{16}H_{25}N_3O_5$
Molecular weight: 339.39
CAS Registry No.: 81801-12-9, 73210-73-8 (hemifumarate)
Merck Index: 10189

SAMPLE
Matrix: blood